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Phosphorus nuclear magnetic resonance study of the rat kidney in vivo

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Phosphorus nuclear magnetic resonance study of the rat kidney in vivo. We show that phosphorus nuclear magnetic resonance (^{31}P-NMR) can be used to study the metabolic state of kidneys in live, anesthetized rats without the need for surgery. To localize signals from the kidney, we used a radiofrequency surface coil in conjunction with the magnetic field profiling technique that is used for "topical magnetic resonance," (TMR). Signals are observed from phosphorus-containing metabolites including ATP and inorganic phosphate, and under certain conditions intracellular pH can be estimated. We suggest that the ratio of free ATP to free ADP is higher than the estimates of 1.5 to 2.0 obtained from freeze-clamping studies. Because of the important roles that ATP, ADP, inorganic phosphate, and pH are believed to play in cellular metabolism, it seems that ^{31}P-NMR could be a powerful clinical tool.

Etude de la résonance magnétique nucléaire du phosphore du rein de rat in vivo. Nous montrons dans ce travail que la résonance magnétique nucléaire du phosphore (^{31}P-NMR) peut être utilisée pour étudier l'état métabolique du rein chez des rats vivant anesthésiés sans avoir recours à la chirurgie. Pour la localisation sur le rein nous utilisons une bobine superficielle de radio-fréquence en association avec le profil de champ magnétique fourni par la technique de résonance magnétique (TMR). Des signaux sont observés à partir de métabolites contenant de phosphore, y compris l'ATP et le phosphate inorganique, et dans certaines conditions le pH intracellulaire peut être évalué. Nous suggérons que le rapport de l'ATP libre à l'ADP libre est plus élevé que l'évaluation de 1,5 à 2 obtenue à partir de reins congelés in vivo. Du fait des rôles importants que l'ATP, l'ADP, le phosphate inorganique et le pH jouent dans le métabolisme cellulaire il semble que la ^{31}P-NMR puisse être un outil clinique puissant.

Phosphorus nuclear magnetic resonance (^{31}P-NMR) is a noninvasive spectroscopic technique that can be used to study the metabolic state of biological tissue (see Refs. 1–4 for reviews). Several phosphorylated metabolites can be detected, including adenosine triphosphate (ATP), phosphocreatine, and inorganic phosphate, and it is also possible to determine intracellular pH. Until recently, the technique was generally applied to isolated tissue preparations, including skeletal muscle [5], perfused heart [6], kidney [7], and liver [8], and to surgically exposed in vivo organs [9]. It would be preferable to detect NMR signals without the need for surgery, so that long-term and truly in vivo studies could be carried out. In this way, the clinical potential of the NMR technique could also be assessed.

Recently, two new techniques were reported from this laboratory for the detection of ^{31}P-NMR spectra from a well-defined region within an animal. The first uses an unusual type of receiver coil, termed a surface coil, which if placed adjacent to a sample, normally detects signals almost exclusively from an approximately disk-shaped region of the sample immediately in front of the coil [10]. The radius and thickness of the disk are both approximately equal to the radius of the particular coil that is used. Surface coils therefore provide a simple method of detecting signals from a localized region close to the surface of a sample. For whole animal studies, they have been used to investigate the metabolism of skeletal muscle and brain [10].

Another technique, topical magnetic resonance (TMR), has been used to detect signals from a localized region deep within a sample. This method ensures that high resolution signals are observed only from a central, approximately spherical region, the radius of which can be varied to suit the sample under investigation. The technique has been successfully used for a study of the liver of live, anesthetized rats [11].

We report here that by combining these two techniques, surface coils and TMR, we have been successful in obtaining high resolution ^{31}P-NMR spectra almost exclusively from the kidney of live, anesthetized rats.

Methods

All spectra were recorded on a spectrometer constructed in this laboratory [12] and interfaced with a Nicolet 1180 computer. The magnet has a field of 4.3 tesla (corresponding to a frequency of 73.8 mHz for ^{31}P), and has a bore of 9.5 cm. The TMR field profiling coils were those described by Gordon et al [11] and were positioned within the normal set of field homogeneity coils. The radiofrequency coil was a two-turn, flat, circular surface coil, wound from copper wire of 1.25 mm in diameter. The inner and outer diameters of the coil were 1.4 cm and 1.9 cm, respectively. Prior to each experiment, the field homogeneity was adjusted using the surface coil to detect ^{1}H signals from the water within the localized region of the animal [13].

Male Wistar rats, 150 to 180 g, were anesthetised with phenobarbitone (4 mg/kg) a few minutes prior to the start of each experiment. The animals were secured in a cradle for placement in the magnet [9]. The position of the kidney was determined by touch, and the NMR surface coil was placed on the dorsal surface of the animal, over the left kidney. The surface coil was isolated from the animal by polytetrafluoroeth-

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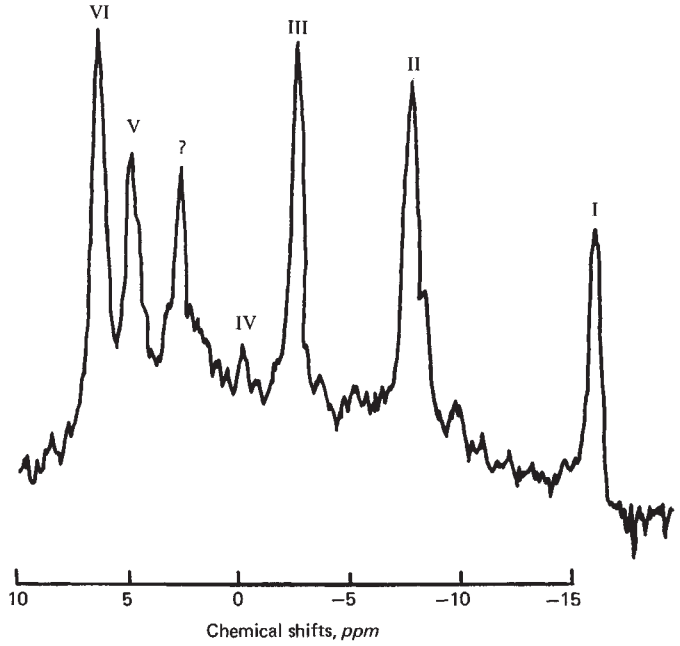

Fig. 1. ^{31}*P-NMR spectrum of a perfused rat kidney.* The spectrum was obtained at 37° C and represents an accumulation of 1800 scans collected over a period of 30 min. The signals are assigned as follows: I, β-phosphate of ATP; II, α-phosphate of ATP, together with contributions from the α-phosphate of ADP, and from NAD/NADH; III, γ-phosphate of ATP, together with a contribution from the β-phosphate of ADP; IV, a barely discernible signal from phosphocreatine; '?' phosphodiester, as yet unidentified; V, inorganic phosphate; VI, sugar phosphate and AMP. Chemical shifts are expressed in parts per million (*ppm*) relative to the phosphocreatine signal. Adapted from Ref. 7.

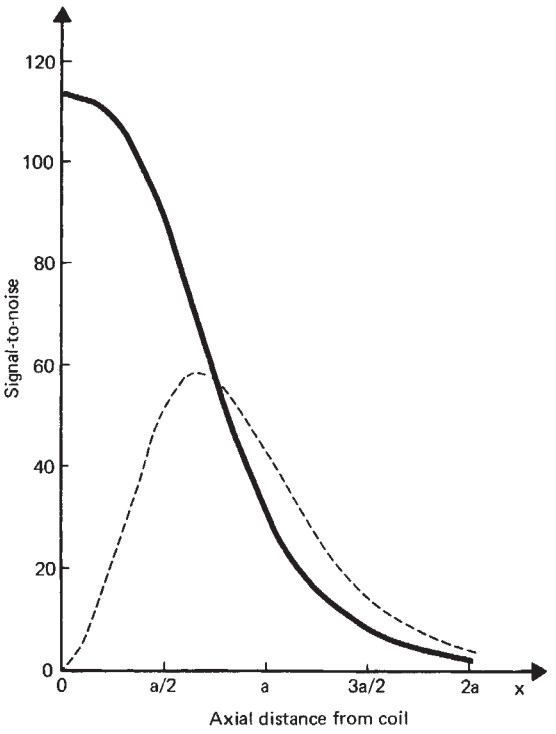

Fig. 3. *The relative signal-to-noise produced by a sample positioned at different axial distances away from a circular surface coil.* The solid curve is for pulses such that the pulse angle is 90° at x = 0, whereas the dotted curve is for pulses such that the pulse angle is 180° at x = 0. The curves were calculated using the assumption that pulses are applied at time intervals equal to the spin-lattice relaxation times (T_1) of the resonances.

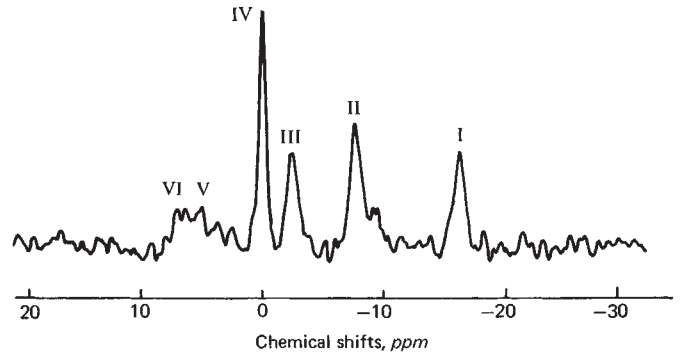

Fig. 2. ^{31}P-*NMR spectrum obtained from a live, anesthetized rat, using the procedure described in "Methods."* For this spectrum, there was no localization provided by TMR; hence the large peak (IV) from phosphocreatine. Assignments are the same as those for Fig. 1. The pulse width was 30 μsec, and the spectrum was accumulated from 64 scans at 2-sec intervals. For this and all subsequent spectra, the technique of convolution difference [14] was used to remove the very broad underlying components of the spectrum, and hence to flatten the baseline. For this particular spectrum, line broadenings of 20 Hz and 200 Hz were used, and the second transform was multiplied by 0.9 before being subtracted from the first.

ylene (PTFE) tape wrapped around the coil. The kidneys of these rats weighed about 0.8 g wet wt.

Results

Figure 1, which shows a ^{31}P-NMR spectrum of a perfused rat kidney, provides the control for the experiments described in this paper. The signals are assigned as shown in the figure and legend. Note that the spectrum contains little, if any, signal from phosphocreatine, which is present at very low concentration in the kidney [7]. In contrast, muscle contains a high concentration of this compound. Therefore, the aim of experiments designed to localize signals from the kidney of a live animal is to obtain a spectrum similar to that shown in Figure 1, containing no signal from phosphocreatine.

A problem in the localization experiments is that there is a layer of muscle between the kidney and skin, which is very close to the surface coil, and therefore generates strong signals, as seen in Figure 2. This spectrum was obtained in the absence of any localization provided by TMR and bears little relationship to the spectrum of Figure 1. One possible method of reducing the contribution from the muscle makes use of the characteristic properties of surface coils.

A circular surface coil produces a radiofrequency field that is strongest close to the coil, but declines rapidly with axial and radial distance away from the coil. It is for this reason that under normal conditions a disk-shaped region immediately in front of the coil generates the dominant contribution to the observed signal. Figure 3 shows how the signal from a sample situated at different points along the axis varies according to axial distance (x) from the coil. The two curves represent two different conditions of irradiation. In Fourier transform NMR, the radiofrequency irradiating field is applied to the sample in the form of pulses, the duration (width) of which affects the nature of the signals that are observed. For normal pulse widths

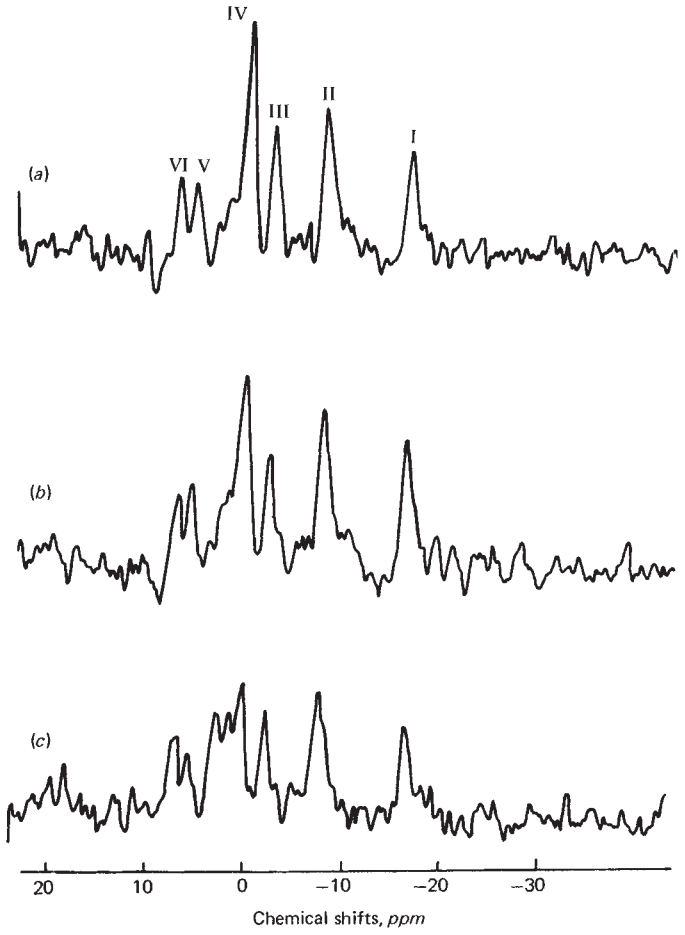

Fig. 4. *^{31}P-NMR spectra obtained from a live, anesthetized rat.* Each spectrum was obtained from 60 scans at 2-sec intervals. *(a)* pulse width, 30 μsec; *(b)* pulse width, 50 μsec; (c) pulse width, 70 μsec. Assignments are the same as for Fig. 1. No localization was provided by TMR. Convolution difference was used as described in the caption to Fig. 2, except that the line broadenings were 30 Hz and 200 Hz.

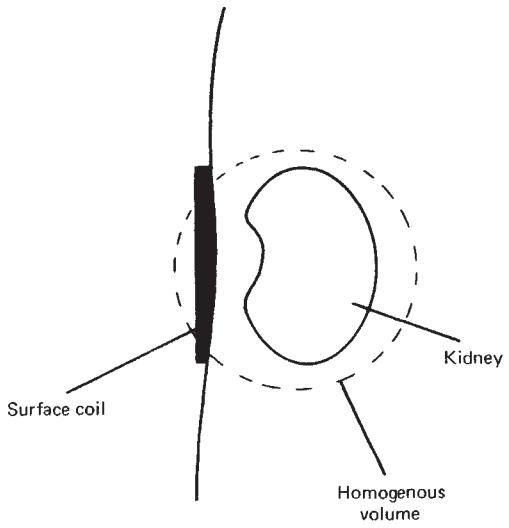

Fig. 5. *Schematic illustration of the coil positioning and the homogeneous region provided by TMR.* The surface coil is positioned adjacent to the body wall over the kidney, and the homogeneous volume, which is bounded by the dotted line, is approximately spherical in shape.

(the solid curve of Fig. 3), the signal drops off sharply at x = a. where a is the radius of the coil. But, if the pulse width is increased to one particular value, one can obtain the dependence shown by the dotted curve of Figure 3. Therefore, by selecting this particular "surface-nulling" pulse width, one could, in principle, eliminate most of the signal from the muscle close to the coil.

In practice, this approach does not work, as shown by the series of spectra in Figure 4. A pulse width of 50 μsec corresponds to a "surface-nulling" pulse, and yet a signal is still observed from phosphocreatine, indicating that muscle still contributes significantly to the spectrum. We conclude that the main reason for the failure of this experiment is that, in increasing the pulse width, we are enhancing the signal from regions further away from the coil, not only in the axial direction, but also in the radial direction. These relatively outlying regions contain a large amount of muscle, and therefore contribute to the phosphocreatine signal.

The signal from the outlying regions can, however, be removed by means of the localization provided by TMR. Figure 5 is a schematic diagram illustrating the approximate position of the kidney relative to the surface coil and to the homogeneous region that is produced by TMR. Figure 6 shows spectra obtained with a pulse width of 50 μsec as the radius of the homogeneous region is reduced, by adjusting the TMR controls, to 0.9 cm and then to 0.6 cm. Gradually, the relative contribution of phosphocreatine declines, and when the radius is 0.6 cm, we obtain a spectrum that is essentially identical to that shown in Figure 1. Thus, we have apparently successfully localized signals from the kidney. When the pulse width was not close to 50 μsec, the localization was not adequate. We conclude that these metabolic measurements on the kidney can be carried out only when TMR is combined with a method that takes advantage of the radiofrequency field profile of the surface coil.

Confirmation that the signals are indeed from the kidney was obtained from experiments in which the renal artery was ligated after a control spectrum from the kidney had been obtained. Figure 7 shows spectra from such an animal that were taken prior to and following the ligation. Figure 7a shows the expected spectrum characteristic of a healthy kidney, whereas Figure 7b shows just the low energy phosphates of the kidney. In the absence of localization, we would observe these low energy phosphates, together with high energy phosphates from other tissues.

Discussion

These results demonstrate that by combining the spatial resolution of TMR and surface coils, ^{31}P-NMR spectra can be obtained completely noninvasively from the kidney of an experimental animal. An important feature of the method is that no surgery is required, for the associated trauma could lead to nonphysiologic alterations of function and metabolism.

What can the ^{31}P-NMR spectra tell us? As discussed elsewhere [1–4], ^{31}P-NMR spectra of biological tissue provide information about the concentrations of *mobile* (and hence, in general, *free*) phosphorus-containing compounds, including inorganic phosphate, phosphocreatine, adenosine triphosphate (ATP), adenosine diphosphate (ADP), and possibly other intermediates such as adenosine monophosphate (AMP) and sugar

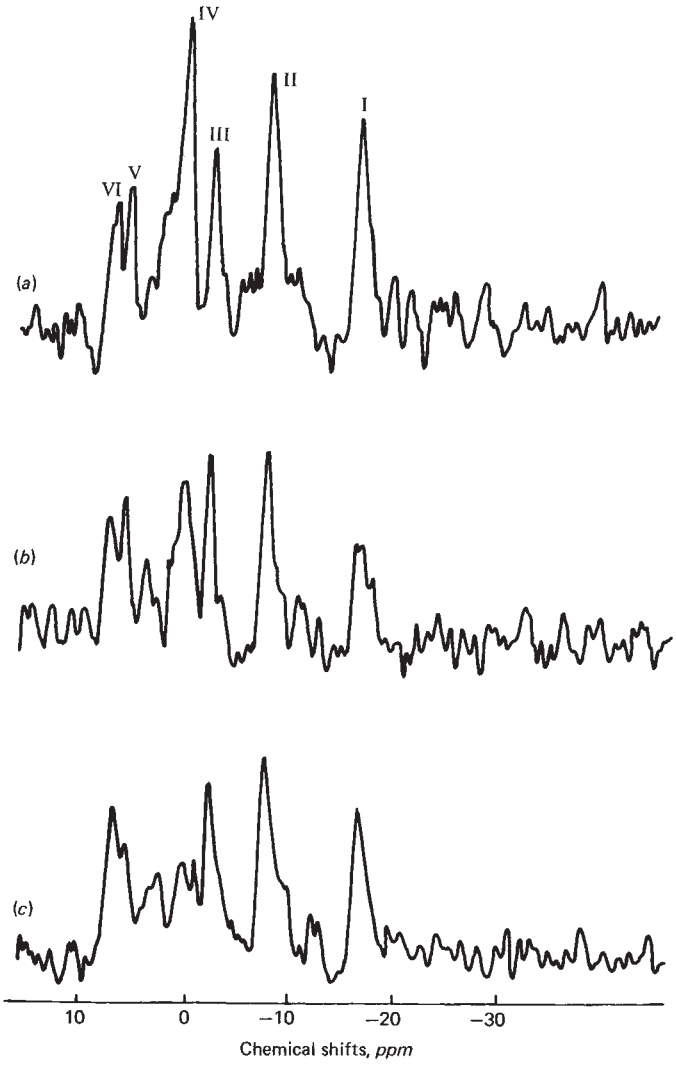

Fig. 6. ^{31}*P-NMR spectra obtained from a live, anesthetized rat.* For each spectrum, the pulse width was 50 μsec. *(a)* No localization was provided by TMR; 60 scans were collected at 2-sec intervals. *(b)* Homogeneous region was reduced to a 0.9-cm radius; 60 scans were collected at 2-sec intervals. *(c)* Homogeneous region was reduced to a 0.6-cm radius; 480 scans were collected at 2-sec intervals. Assignments are like those for Fig. 1, and convolution difference was the same as that used for Fig. 4.

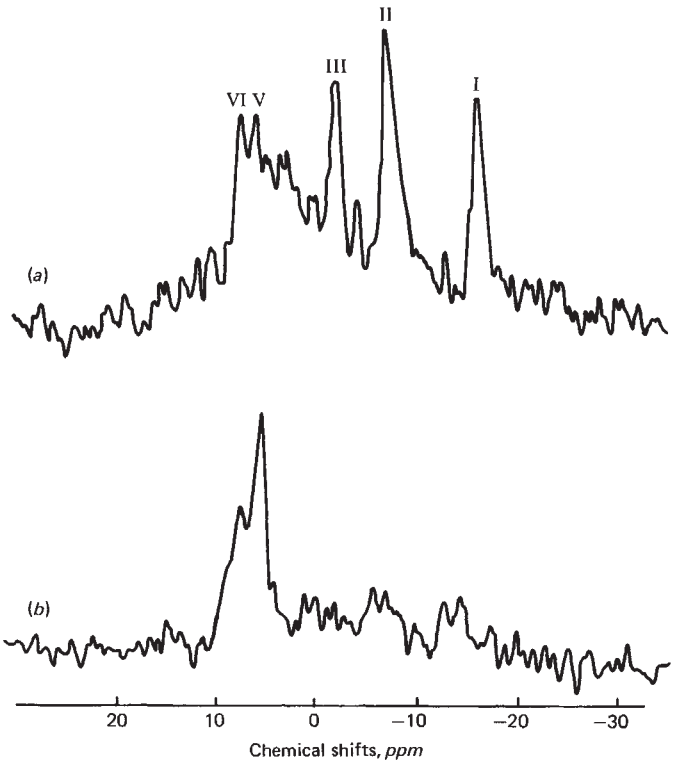

Fig. 7. ^{31}*P-NMR spectra obtained from a live, anesthetized rat.* For both spectra, the pulse width was 50 μsec, and the radius of the homogeneous region was 0.6 cm. *(a)* prior to ligation, 1088 scans were obtained at 2-sec intervals; *(b)* following ligation, 544 scans were obtained at 2-sec intervals. The convolution difference procedure was the same as that used for Fig. 4.

phosphates. As the technique is insensitive, signals can be observed only from compounds present at concentrations above 0.2 to 0.5 mM. In addition, the tissue pH can be monitored from the frequency of the inorganic phosphate signal, and information is also available from the ATP resonance frequencies about the level of free magnesium ions.

The detection only of mobile compounds contrasts with the classical freeze-clamp and extraction procedures, which measure the total cellular amounts of the metabolites. In addition, NMR is not subject to freeze-clamp artifacts, and for these reasons NMR can provide important new information about the environments and concentrations of metabolites in the kidney. Previous freeze-clamp studies on the ATP/ADP ratio in the intact kidney yield values in the range 1.5 to 2.0 [15–17]. Provided that the appropriate controls are performed [1], the ATP/ADP ratio can be calculated from the ^{31}P-NMR spectra, from the ratio of the areas of the peaks labeled I and III in the spectra. This is because ATP and ADP both contribute to peak III, but only ATP contributes to peak I. From the spectra obtained in this study, it is reasonable to conclude that the ATP/ADP ratio detected by NMR is much greater than the estimates of 1.5 to 2.0 obtained from the freeze-clamping technique. A similar conclusion can be obtained for the perfused kidney from spectra of the type shown in Fig. 1. This suggests either that the freeze-clamping and subsequent extraction of ATP and ADP may be inaccurate in this tissue where the half-life of ATP in the cortex is less than 1.5 sec [17], or that a large amount of ADP may be bound or compartmentalized, as proposed by Veech et al in other tissues [18], from kinase equilibrium values. The amount of inorganic phosphate in the organ is difficult to quantitate at present because 2,3-diphosphoglycerate in the blood also produces signals in this region of the spectrum. But, in conditions where the tissue inorganic phosphate is high, this peak is readily detected and assigned, and pH can therefore be ascertained.

The critical roles that ATP, ADP, inorganic phosphate, and pH are believed to play in cellular metabolism and viability suggest that this technique will be a powerful clinical tool. Further applications of the present technique will allow the characterization of the effect of both acute and chronic alter-

ations of renal function on tissue pH and phosphorylated metabolic intermediates. It is hoped that these studies will not only advance our knowledge of renal physiology but also evaluate the diagnostic potential of the technique.

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